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Anti-integrin $\alpha\beta6$ antibody as a diagnostic marker for pediatric patients with ulcerative colitis

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Anti-Integrin $\alpha v \beta 6$ Antibody as a Diagnostic Marker for Pediatric Patients With Ulcerative Colitis



Ulcerative colitis (UC) is a multifactorial chronic disorder with a high prevalence worldwide. UC affects patients in all age groups; however, pediatric-onset UC is generally more severe than adult-onset UC.¹ To avoid long-term unfavorable outcomes, such as growth failure, early and proper diagnosis with subsequent treatment is needed for pediatric UC patients. Gastrointestinal endoscopy is the criterion standard for a diagnosis of UC, but the procedure is invasive for pediatric patients, often requiring the use of general anesthesia. Thus, a non-invasive and disease-specific marker for diagnosing pediatric UC is strongly required.

We recently identified anti-integrin $\alpha v \beta 6$ antibody in adult UC patients with very high sensitivity and specificity, indicating that this autoantibody can be a useful diagnostic marker for UC in adult patients.² Here, we examined the presence and diagnostic value of this antibody in pediatric patients with inflammatory bowel disease (IBD), including UC.

We enrolled 261 pediatric IBD patients (131 UC, 95 Crohn's disease [CD], 10 IBD—unclassified [IBD-U], 25 primary immunodeficiency [PID] with IBD), 40 non-IBD patients (13 PID without IBD and 27 non-IBD enterocolitis), and 28 non-enterocolitis control subjects (Supplementary Figure 1A). Details of patients and control subjects are provided in the Supplementary Methods.

A total of 124 of the 131 UC patients (94.7%) had immunoglobulin (Ig) G antibodies against integrin $\alpha v \beta 6$ based on a cutoff value of the mean plus 3 standard deviations (optical density = 0.2525) for the non-enterocolitis control subjects. Moreover, these IgG antibodies were detected in 31/95 (32.6%) CD, 2/10 (20.0%) IBD-U, 2/25 (8.0%) PID with IBD, 0/13 (0%) PID without IBD, and 1/27 (3.7%) non-IBD enterocolitis patients, and in 1/28 (3.6%) non-enterocolitis control subjects (Figure 1A). The sensitivity and specificity of anti-integrin $\alpha v \beta 6$ IgG autoantibodies in UC patients were, respectively, 94.7% (124/131) and 81.3% (161/198). On the other hand, the specificity of anti-integrin $\alpha v \beta 6$ IgG autoantibodies in the diagnosis of UC against CD was 67.4% (64/95).

Next, we examined the IgG subclasses and autoantibody isotypes. Among 47 randomly selected antibody-positive UC patients, 47 (100.0%), 38 (80.9%), 14 (29.8%), 26 (55.3%), 9 (19.1%), 28 (59.6%), and 12 (25.5%) had IgG1, IgG2, IgG3, IgG4, IgM, IgA, and IgE antibodies, respectively (Supplementary Figure 1B and C). These data are similar to those found in the adult patients previously reported with UC.² Moreover, the subclasses and isotypes of anti-integrin $\alpha v \beta 6$ antibodies in patients with CD or PID with IBD were similar to those of patients with UC (Supplementary Figure 1B and C).

Previously, we showed that IgG of adult UC patients blocked integrin $\alpha v \beta 6$ -fibronectin binding through an Arg-Gly-Asp (RGD) tripeptide motif (Supplementary

Figure 2A). Consistently, a solid-phase binding assay showed that IgG of 45/47 (95.7%) antibody-positive pediatric UC patients blocked integrin $\alpha v \beta 6$ -fibronectin binding (Figure 1B). The blocking activity of IgG was correlated with the anti-integrin $\alpha v \beta 6$ antibody titers ($r = 0.83$; $P < 0.001$) (Supplementary Figure 2B). Furthermore, RGD-serine (RGDS) peptides inhibited the binding of IgG to integrin $\alpha v \beta 6$ in a dose-dependent manner, whereas Arg-Gly-Glu-Ser (RGES) peptides did not block such binding (Supplementary Figure 2C). Moreover, IgG of 23/25 (92.0%) CD, 2/2 (100%) IBD-U, and 2/2 (100%) PID with IBD patients with anti-integrin $\alpha v \beta 6$ antibody inhibited integrin $\alpha v \beta 6$ -fibronectin binding (Figure 1B), and RGDS peptides blocked the binding of IgG of these patients to integrin $\alpha v \beta 6$ (Supplementary Figure 2D and E). In contrast, IgG of non-IBD enterocolitis patients and non-enterocolitis control subjects with anti-integrin $\alpha v \beta 6$ antibody neither blocked integrin $\alpha v \beta 6$ -fibronectin binding nor competed with RGDS peptides on binding to integrin $\alpha v \beta 6$ (Figure 1B and Supplementary Figure 2E).

Notably, a considerable number of CD patients in this study (31/95, 32.6%) (Figure 1A) had anti-integrin $\alpha v \beta 6$ antibodies, in contrast to its low number in adult CD patients (5/71, 7.0%).² Moreover, these antibodies of CD patients showed characteristics similar to those of UC patients, including IgG subclasses, isotypes, blocking activity on integrin $\alpha v \beta 6$ -fibronectin binding, and inhibition of RGDS peptide binding to integrin $\alpha v \beta 6$. Therefore, we focused on the clinical characteristics of our pediatric CD patients. We classified various endoscopic and pathologic findings of the CD patients into typical UC and typical CD findings, as described in the Supplementary Methods (Supplementary Figure 1D).^{3,4} The average total number of typical UC findings was significantly higher (3.23 vs 0.61; $P < 0.001$) whereas the average number of typical CD findings was significantly lower (2.00 vs 3.02; $P < 0.001$) in antibody-positive CD patients than in antibody-negative CD patients (Figure 1C and Supplementary Figure 1E). This result suggests that pediatric CD patients positive for anti-integrin $\alpha v \beta 6$ antibody have UC-like characteristics. Of note, in pediatric IBD, the diagnosis of UC may change to CD.⁵ Indeed, 15 of the 95 CD patients in this study had been initially

Abbreviations used in this paper: CD, Crohn's disease; IBD, inflammatory bowel disease; IBD-U, inflammatory bowel disease—unclassified; PID, primary immunodeficiency; UC, ulcerative colitis.

Most current article

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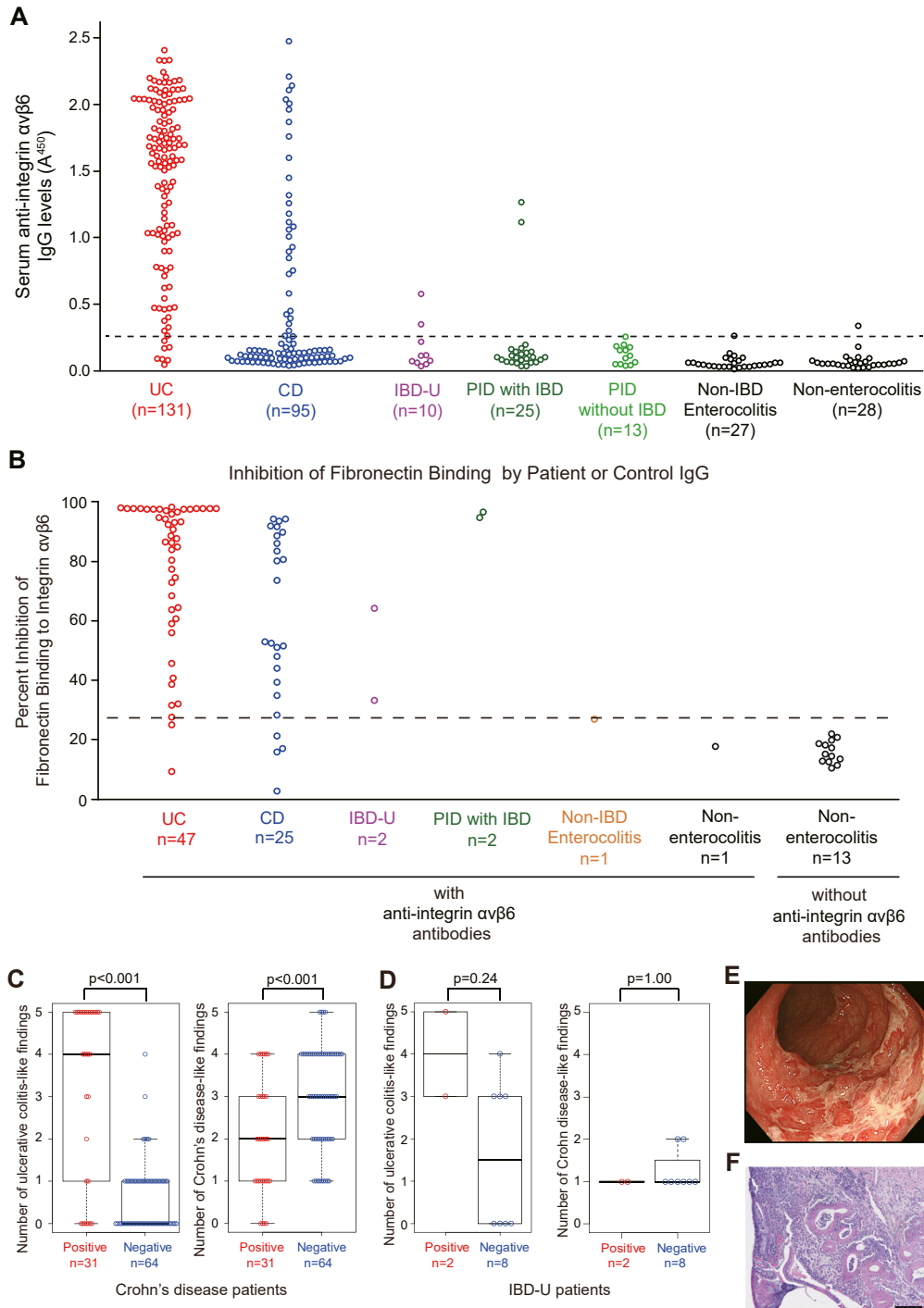


Figure 1. (A) Serum IgG antibodies against integrin $\alpha v \beta 6$ were detected with the use of an enzyme-linked immunosorbent assay. The sera of 131 pediatric patients with UC, 95 pediatric patients with CD, 10 patients with IBD-U, 25 patients with PID with IBD, 13 patients with PID without IBD, 27 patients with non-IBD enterocolitis, and 28 patients without enterocolitis (control) were examined. The cutoff optical density (OD), defined as the mean plus 3 standard deviations of the sera of non-enterocolitis patients, is indicated by a *dashed line*. (B) Inhibition of integrin $\alpha v \beta 6$ –fibronectin binding by IgG of anti-integrin $\alpha v \beta 6$ antibody–positive pediatric patients according to solid-phase binding assay. The cutoff OD, defined as the mean plus 3 standard deviations of IgG from the non-enterocolitis control patients, is indicated by a *dashed line*. (C) Comparison of endoscopic and pathologic findings between pediatric CD patients with and without anti-integrin $\alpha v \beta 6$ antibodies. We classified various endoscopic and pathologic findings into typical UC findings or typical CD findings as previously reported (Supplementary Figure 1D). (D) Comparison of endoscopic and pathologic findings between pediatric IBD-U patients with and without anti-integrin $\alpha v \beta 6$ antibodies. (E) Colonoscopy of a patient with cytotoxic T-lymphocyte-associated antigen 4 haploinsufficiency showed continuous, circumferential, and diffuse erythema and ulceration in the rectum to the transverse colon and descending colon. (F) Pathologic images of the biopsies of the same patient showed intraepithelial infiltration of neutrophils and eosinophils (cryptitis, crypt abscess). Hematoxylin and eosin staining; scale bar = 100 μm .

diagnosed with UC, which was later changed to CD; 13 of these 15 patients had anti-integrin $\alpha v\beta 6$ antibodies (86.7%).

IBD-U patients with anti-integrin $\alpha v\beta 6$ antibodies had UC-like findings, whereas those without anti-integrin $\alpha v\beta 6$ antibodies were less likely to have UC-like findings (Figure 1D).

One of the 2 PID with IBD patients who were positive for anti-integrin $\alpha v\beta 6$ antibodies (Figure 1A) had cytotoxic T-lymphocyte-associated antigen 4 haploinsufficiency. The colonoscopy of this patient showed continuous circumferential and diffuse inflammation in the rectum similar to that found in UC (Figure 1E). Biopsies showed crypt abscesses, which is also a pathology resembling UC (Figure 1F). The other patient had activated phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit delta syndrome. Colonoscopy showed lymphoid follicular hyperplasia with erythema continuous from the lower rectum, which was previously reported as an early sign of UC.⁶ These 2 diseases are characterized by T-cell dysfunction and presence of various autoantibodies.^{7,8} Thus, such T-cell dysregulation might have induced the production of anti-integrin $\alpha v\beta 6$ antibodies along with development of UC-like colitis in the 2 patients.

In conclusion, we found that anti-integrin $\alpha v\beta 6$ antibodies with characteristics similar to those in adult UC patients are present in pediatric UC patients with high sensitivity. Moreover, patients with CD, IBD-U, or PID with IBD, who were positive for anti-integrin $\alpha v\beta 6$ antibody had UC-like colitis. These data suggest the usefulness of this antibody not only for making an appropriate diagnosis of pediatric UC but also for establishing a novel classification of pediatric IBD, particularly by combining the data of anti-*Saccaromyces cerevisiae* antibodies and anti-neutrophil cytoplasm antibodies. Finally, we found that IgG of most of the pediatric UC patients with anti-integrin $\alpha v\beta 6$ antibodies blocked integrin $\alpha v\beta 6$ -fibronectin binding through an RGD motif. Previous studies showed that integrin $\alpha v\beta 6$ may play an important role in epithelial barrier function and mucosal healing.^{9,10} Taken together, this autoantibody may affect the mucosal barrier function in UC. However, its significance in vivo is unknown; further investigation is needed.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at <https://doi.org/10.1053/j.gastro.2022.06.026>.

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CRedit Authorship Contributions

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Hiroshi Nihira, MD (Conceptualization: Equal; Data curation: Equal; Formal analysis: Equal; Investigation: Equal; Supervision: Equal; Visualization: Equal; Writing – original draft: Equal; Writing – review & editing: Equal).

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Conflicts of interest

The authors disclose no conflicts.

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Data Transparency

The authors confirm that the data supporting the findings of this study are available within the article and supplementary materials.

Supplementary Methods

Patients

Ulcerative colitis (UC) was diagnosed on the basis of clinical symptoms, endoscopic findings, histologic findings, and the exclusion of potential differential diagnoses.^{1–3} Diseases other than UC were diagnosed on the basis of the Japanese diagnostic criteria. The clinical characteristics of the patients are summarized in [Supplementary Figure 1A](#) and [Supplementary Table 1](#). Serum samples were collected from 12 participating institutions in Japan from 2011 to 2021. For subclass and isotype analysis of antibodies and the solid-phase integrin $\alpha\text{v}\beta\text{6}$ binding assay, patients positive for anti-integrin $\alpha\text{v}\beta\text{6}$ antibodies with sufficient serum were randomly selected; some patients without anti-integrin $\alpha\text{v}\beta\text{6}$ antibodies were also selected for this analysis. All serum samples were stored at -30°C or -80°C until assayed. The experiments were performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (protocol numbers R2443, G0457, G0729, G1091, and G1118). Informed consent was obtained from the parents of children, and signed youth consent was obtained from patients when appropriate.

Analyses of Endoscopic and Pathologic Findings

Endoscopic findings were evaluated by pediatricians or gastroenterologists blinded to the anti-integrin $\alpha\text{v}\beta\text{6}$ antibody titer. We classified various endoscopic and pathological findings of the patients with Crohn's disease (CD) or inflammatory bowel disease—unclassified into typical UC and typical CD findings as previously reported ([Supplementary Figure 1D](#)).^{3,4} The total number of these findings was calculated for each patient.

Enzyme-Linked Immunosorbent Assay

The human recombinant integrin $\alpha\text{v}\beta\text{6}$ protein (IT6-H52E1; Acrobiosystems, Newark, NJ, USA) was used as an antigen. The detection of serum immunoglobulin (Ig) G against integrin $\alpha\text{v}\beta\text{6}$ protein was performed with the use of an enzyme-linked immunosorbent assay (ELISA) Starter Accessory Kit (E101; Bethyl Laboratories, Montgomery, TX, USA) according to the manufacturer's instructions. Briefly, microtiter plates were coated with $100\ \mu\text{L}$ of $2\ \mu\text{g}/\text{mL}$ recombinant protein in $50\ \text{mmol}/\text{L}$ carbonate-bicarbonate (pH 9.6) and incubated at 4°C overnight, blocked with $50\ \text{mmol}/\text{L}$ Tris-buffered saline solution (TBS) containing 1% bovine serum albumin (BSA) for 30 minutes at room temperature, and incubated with $100\ \mu\text{L}$ serum or purified IgG diluted by TBS containing 0.05% Tween 20 and 1% BSA (1:100) for 60 minutes at room temperature. After 5 washes with TBS containing 0.05% Tween 20 (wash solution), the plates were incubated with $100\ \mu\text{L}$ goat anti-human IgG antibody conjugated with horseradish peroxidase (HRP) (1:50,000; ab6759; Abcam, Cambridge, UK) at room temperature for 60 minutes. After another 5 washes, the bound reactants were detected by means of incubation

for 7 minutes with 3,3',5,5'-tetramethylbenzidine (TMB). The absorbance was measured at 450 nm. ELISA was performed in the presence of MgCl_2 and CaCl_2 ($1\ \text{mmol}/\text{L}$ each).⁵

To evaluate the autoantibody subclasses, we used anti-human IgG1, IgG2, IgG3, and IgG4 conjugated with HRP (1:10,000; BS-AP006, 1:2,000; BS-AP007, BS-AP008, and BS-AP009, respectively; The Binding Site, Birmingham, UK) as secondary antibodies. To examine autoantibody isotypes, we used anti-human IgM, IgA, and IgE conjugated with HRP (1:100,000 A80-100P, 1:50,000 A80-102P, and 1:1,000 A80-108P, respectively; Bethyl Laboratories) as secondary antibodies.

To examine whether the Arg-Gly-Asp (RGD) peptide inhibited the binding of patient IgG to integrin $\alpha\text{v}\beta\text{6}$, we added the RGD-Ser peptide (A9041; Sigma-Aldrich, St Louis, MO, USA) or the Arg-Gly-Glu-Ser (RGES) peptide (A5686, Sigma-Aldrich) to the purified IgG at concentrations of 6.75, 12.5, 25, 50, and $100\ \mu\text{g}/\text{mL}$ before incubation.

Preparation of Human IgG

IgG from the sera of patients was purified with the use of Ab-Rapid SPiN EX (P-014; ProteNova, Higashikagawa, Japan) according to the manufacturer's instructions. The purified IgG was stored at -30°C . The IgG recovery rate from the sera was confirmed to be $>90\%$ in our previous study.^{5,6}

Solid-Phase Integrin $\alpha\text{v}\beta\text{6}$ -Binding Assay

The solid-phase integrin $\alpha\text{v}\beta\text{6}$ binding assay was performed according to a previously described method, with minor modifications, using the ELISA Starter Accessory Kit (E101, Bethyl Laboratories).^{5,7} Briefly, a 96-well microtiter plate was coated with $100\ \mu\text{L}/\text{well}$ of $2\ \mu\text{g}/\text{mL}$ fibronectin (FC010; MilliporeSigma, Burlington, MA, USA) overnight at 4°C and blocked for 30 minutes at room temperature. Thereafter, $100\ \mu\text{L}$ diluted patient or control IgG (1:10) premixed with $0.2\ \mu\text{g}/\text{mL}$ His-tagged integrin $\alpha\text{v}\beta\text{6}$ (IT6-H52E1, Acrobiosystems) at 4°C overnight was incubated for 60 minutes at room temperature. After 5 washes with wash solution, a rabbit anti-His tag polyclonal antibody (1:1,000; PM032; MBL, Nagoya, Japan) was added, followed by incubation at room temperature for 60 minutes. After another 5 washes with wash solution, an anti-rabbit IgG HRP-conjugated secondary antibody (1:1,000; code no. 458, MBL) was added, followed by incubation at room temperature for 60 minutes. After 5 washes with wash solution, the bound reactants were detected by means of reacting with TMB and incubation for 10 minutes. The absorbance was measured at 450 nm. This assay was also performed in the presence of MgCl_2 and CaCl_2 ($1\ \text{mmol}/\text{L}$ each). To calculate the inhibition rate, we used control wells coated with fibronectin and incubated with integrin $\alpha\text{v}\beta\text{6}$, but not with purified IgG. The inhibition rate was calculated with the use of the following formula: (control optical density [OD] – sample OD)/control OD. The cutoff OD was defined as the mean plus 3 standard deviations of the IgG from non-enterocolitis patients.

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Histopathologic Examination

Unstained slides were stained with hematoxylin-eosin with the use of standard methods.

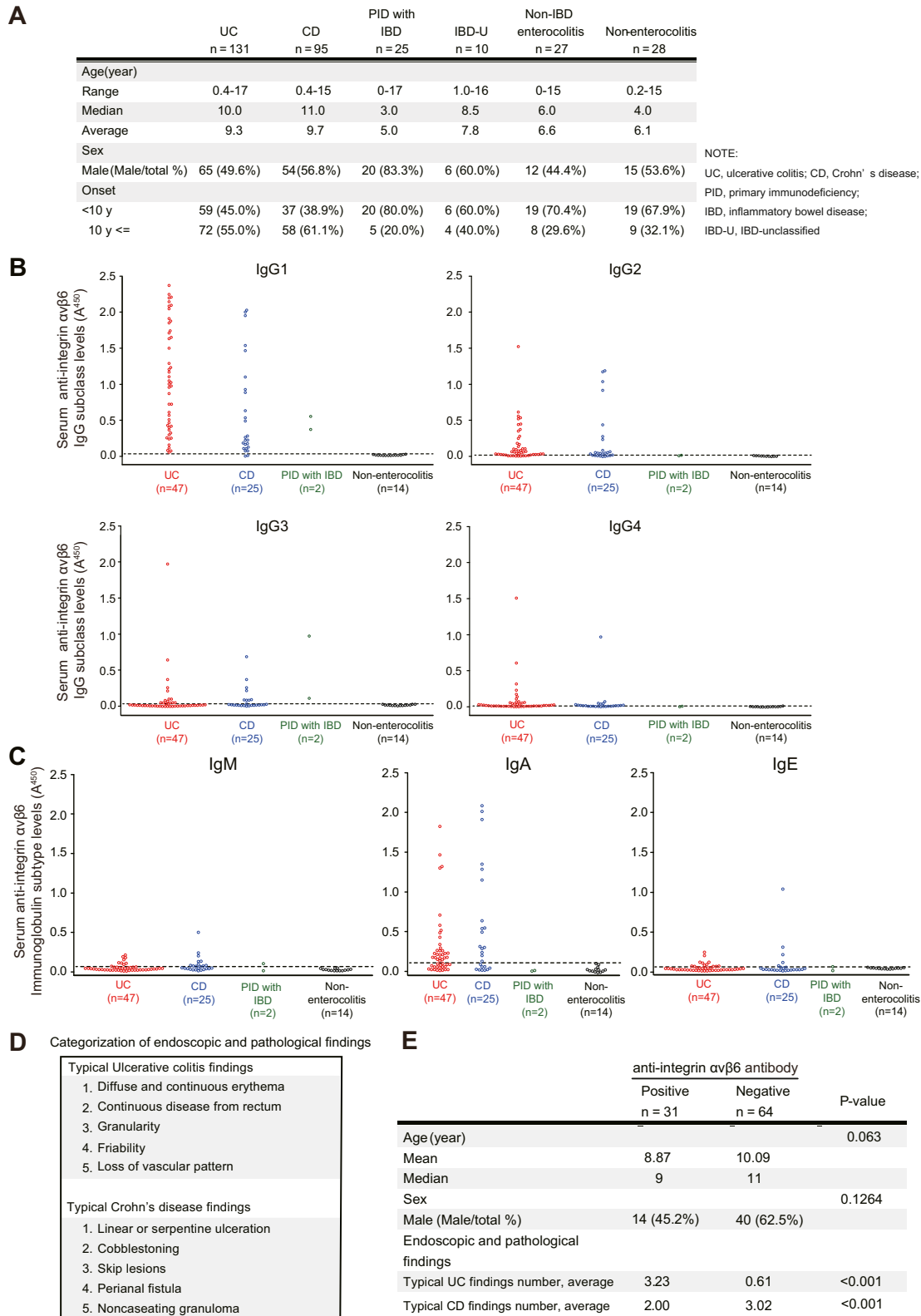
Statistical Analyses

Statistical analyses were performed with the use of GraphPad Prism version 9.1.2 (GraphPad, La Jolla, CA, USA) or R version 3.6.3. The associations between categorical variables were evaluated by means of Fisher exact test, and continuous variables were compared by means of Mann-Whitney *U* tests. The correlation between IgG antibody titers against integrin $\alpha v \beta 6$ and the blocking of integrin $\alpha v \beta 6$ -fibronectin binding was evaluated by means of the

Pearson product moment correlation. A *P* value of <0.05 was considered to indicate statistical significance.

Supplementary References

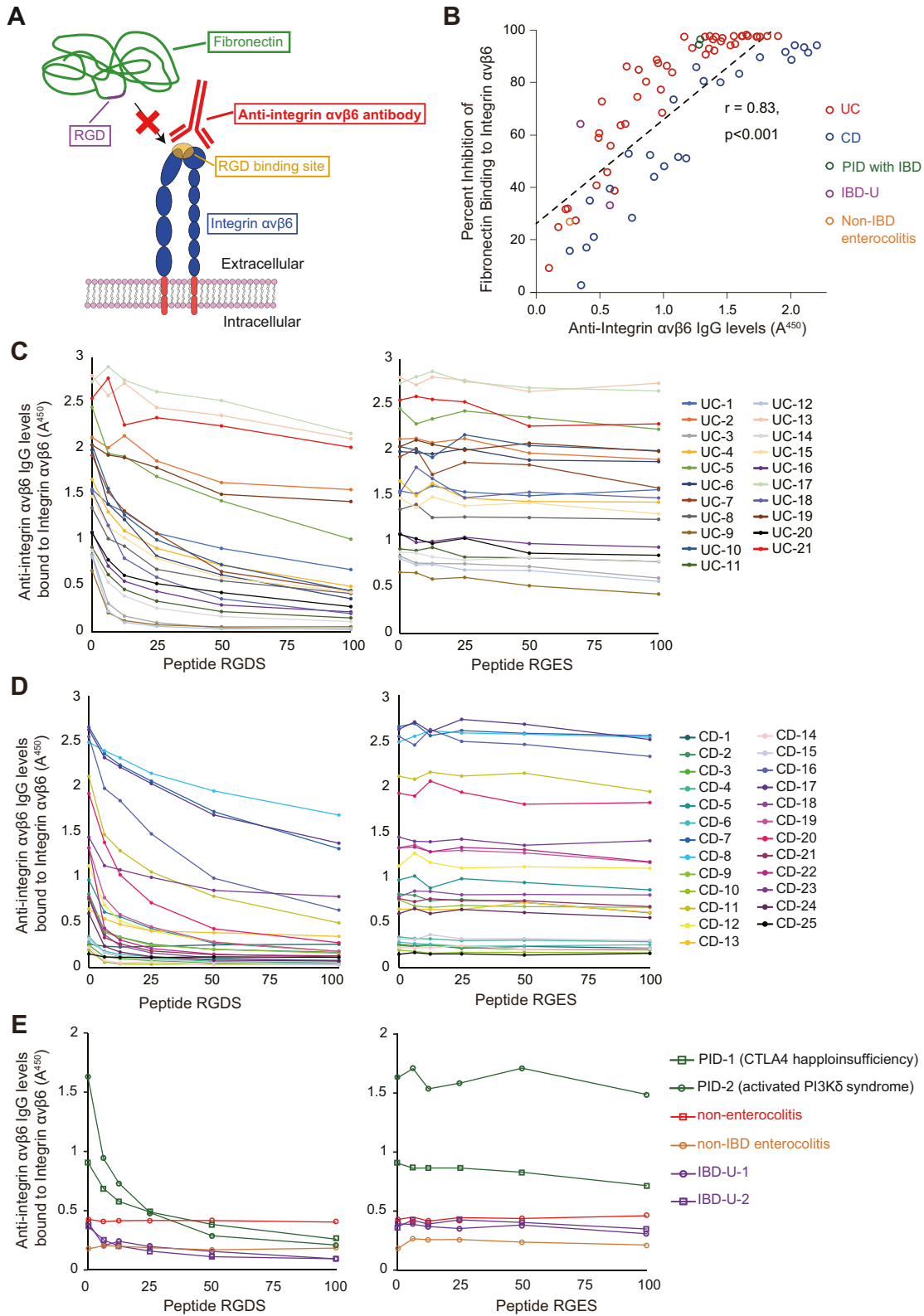
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Supplementary Figure 1. (A) Characteristics of patients. (B) Immunoglobulin (Ig) G subclasses of the anti-integrin $\alpha v \beta 6$ antibodies quantified by enzyme-linked immunosorbent assay (ELISA). Serum samples were incubated with integrin $\alpha v \beta 6$, followed by incubation with horseradish peroxidase (HRP)-conjugated antibodies specific for each human IgG subclass. The dashed line indicates the cutoff optical density, defined as the mean plus 3 standard deviations of the sera of non-enterocolitis patients. (C) Isotypes of anti-integrin $\alpha v \beta 6$ antibodies quantified by means of ELISA. Serum samples were incubated with integrin $\alpha v \beta 6$, followed by incubation with HRP-conjugated antibodies specific for human IgM, IgA, or IgE. For analyses of (B) and (C), patients with anti-integrin $\alpha v \beta 6$ antibodies and sufficient serum were selected. (D) Categorization of endoscopic and pathologic findings.^{3,4} (E) Comparison of endoscopic and pathologic characteristics between patients with Crohn's disease who were positive vs negative for anti-integrin $\alpha v \beta 6$ antibody.

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Supplementary Figure 2. (A) Schematic illustration of integrin $\alpha v \beta 6$ binding to its ligands (eg, fibronectin) by recognizing an Arg-Gly-Asp (RGD) sequence motif, which is inhibited by anti-integrin $\alpha v \beta 6$ antibodies.⁵ (B) Titers of IgG against integrin $\alpha v \beta 6$ correlated with the blocking activity on integrin $\alpha v \beta 6$ -fibronectin binding ($r = 0.83$; $P < 0.001$). (C–E) Arg-Gly-Asp-Ser (RGDS), but not Arg-Gly-Glu-Ser (RGES), peptide impaired the binding of IgG of antibody-positive pediatric (C) UC, (D) Crohn's disease (CD), and (E) other patients to integrin $\alpha v \beta 6$ in a dose-dependent manner. The binding of IgG of non-inflammatory bowel disease (IBD) enterocolitis and non-enterocolitis patient to integrin $\alpha v \beta 6$ was not inhibited by RGDS peptides. IBD-U, inflammatory bowel disease—unclassified; PID, primary immunodeficiency.

Supplementary Table 1. Characteristics of Patients With Ulcerative Colitis (UC)

Sample	Age		Sex	Diagnosis	Extent of disease ¹	PUCAI	Anti-integrin α v β 6 antibody
	y	mo					
UC-1	11		M	UC	E4: pancolitis	0	+
UC-2	5		M	UC	E4: pancolitis	40	+
UC-3	11		F	UC	E4: pancolitis	0	+
UC-4	9		F	UC	E4: pancolitis	0	+
UC-5	10		M	UC	E4: pancolitis	0	+
UC-6	6		M	UC	E1: proctitis	0	-
UC-7	11		F	UC	E4: pancolitis	0	+
UC-8	7		F	UC	E4: pancolitis	0	+
UC-9	9		M	UC	E4: pancolitis	0	+
UC-10	14		F	UC	E4: pancolitis	20	+
UC-11	8		M	UC	E4: pancolitis	0	+
UC-12	3	0	M	UC	E4: pancolitis	10	+
UC-13	15		F	UC	E4: pancolitis	0	+
UC-14	9		M	UC	E4: pancolitis	0	+
UC-15	3	0	M	UC	E4: pancolitis	35	+
UC-16	12		F	UC	E4: pancolitis	0	+
UC-17	11		M	UC	E4: pancolitis	0	+
UC-18	13		F	UC	E4: pancolitis	0	-
UC-19	1	0	M	UC	E4: pancolitis	0	+
UC-20	3	0	M	UC	E4: pancolitis	40	+
UC-21	7		F	UC	E4: pancolitis	0	+
UC-22	11		M	UC	E4: pancolitis	10	+
UC-23	13		M	UC	E4: pancolitis	0	+
UC-24	13		M	UC	E2: left-side colitis	0	+
UC-25	7		M	UC	E4: pancolitis	20	+
UC-26	7		F	UC	E4: pancolitis	0	+
UC-27	9		M	UC	E4: pancolitis	0	-
UC-28	13		F	UC	E4: pancolitis	5	+
UC-29	15		F	UC	E4: pancolitis	0	+
UC-30	13		F	UC	E4: pancolitis	5	+
UC-31	0	5	F	UC	E2: left-side colitis	0	+
UC-32	13		M	UC	E4: pancolitis	0	+
UC-33	2	11	F	UC	E4: pancolitis	5	+
UC-34	12		F	UC	E4: pancolitis	0	+
UC-35	2	6	M	UC	E4: pancolitis	0	+
UC-36	11		M	UC	E4: pancolitis	0	+
UC-37	11		M	UC	E4: pancolitis	40	+
UC-38	13		F	UC	E4: pancolitis	30	+

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 Anti-Integrin $\alpha v \beta 6$ Antibody in Pediatric UC 1097.e6

Supplementary Table 1. Continued

Sample	Age		Sex	Diagnosis	Extent of disease ¹	PUCAI	Anti-integrin $\alpha v \beta 6$ antibody
	y	mo					
UC-39	3	0	F	UC	E4: pancolitis	10	+
UC-40	13		M	UC	E4: pancolitis	0	+
UC-41	14		F	UC	E4: pancolitis	0	+
UC-42	10		M	UC	E4: pancolitis	0	+
UC-43	15		F	UC	E4: pancolitis	0	+
UC-44	12		M	UC	E4: pancolitis	0	+
UC-45	15		F	UC	E2: left-side colitis	0	+
UC-46	8		M	UC	E1: proctitis	10	+
UC-47	12		F	UC	E4: pancolitis	0	+
UC-48	3	8	M	UC	E4: pancolitis	5	+
UC-49	14		M	UC	E3: extensive	0	+
UC-50	14		M	UC	E4: pancolitis	0	+
UC-51	13		M	UC	E4: pancolitis	0	+
UC-52	10		M	UC	E4: pancolitis	5	+
UC-53	9		F	UC	E2: left-side colitis	0	+
UC-54	4		F	UC	E4: pancolitis	0	+
UC-55	12		F	UC	E2: left-side colitis	10	+
UC-56	3	0	M	UC	E4: pancolitis	0	+
UC-57	2	0	M	UC	E4: pancolitis	0	+
UC-58	5		F	UC	E4: pancolitis	45	+
UC-59	3	0	F	UC	E4: pancolitis	0	-
UC-60	8		M	UC	E4: pancolitis	0	+
UC-61	4		F	UC	E4: pancolitis	0	+
UC-62	9		F	UC	E4: pancolitis	0	+
UC-63	9		F	UC	E4: pancolitis	0	+
UC-64	11		F	UC	E4: pancolitis	0	+
UC-65	11		M	UC	E4: pancolitis	0	+
UC-66	13		F	UC	E3: extensive	20	+
UC-67	11		F	UC	E4: pancolitis	0	+
UC-68	11		F	UC	E4: pancolitis	0	+
UC-69	13		M	UC	E4: pancolitis	35	+
UC-70	12		F	UC	E4: pancolitis	5	+
UC-71	8		F	UC	E4: pancolitis	0	+
UC-72	13		F	UC	Right-side colitis and proctitis	0	+
UC-73	11		F	UC	E4: pancolitis	0	+
UC-74	12		F	UC	E4: pancolitis	0	+
UC-75	5		F	UC	E4: pancolitis	0	+
UC-76	14		M	UC	E4: pancolitis	55	+

Supplementary Table 1. Continued

Sample	Age		Sex	Diagnosis	Extent of disease ¹	PUCAI	Anti-integrin $\alpha\text{v}\beta\text{6}$ antibody
	y	mo					
UC-77	14		M	UC	E4: pancolitis	0	+
UC-78	12		F	UC	E4: pancolitis	0	+
UC-79	13		M	UC	E1: proctitis	0	+
UC-80	10		F	UC	E4: pancolitis	0	+
UC-81	11		F	UC	E4: pancolitis	0	+
UC-82	13		M	UC	E4: pancolitis	0	+
UC-83	14		F	UC	E4: pancolitis	0	+
UC-84	13		M	UC	E2: left-side colitis	0	+
UC-85	15		M	UC	E4: pancolitis	65	+
UC-86	12		M	UC	E4: pancolitis	0	+
UC-87	17		F	UC	E4: pancolitis	0	+
UC-88	2	0	M	UC	E4: pancolitis	0	+
UC-89	9		M	UC	E4: pancolitis	70	+
UC-90	1	0	F	UC	E4: pancolitis	0	+
UC-91	9		F	UC	E2: left-side colitis	35	+
UC-92	11		M	UC	E2: left-side colitis	20	+
UC-93	11		M	UC	E4: pancolitis	80	+
UC-94	5		M	UC	E2: left-side colitis	5	–
UC-95	2	6	F	UC	E4: pancolitis	0	+
UC-96	2	10	F	UC	E4: pancolitis	0	+
UC-97	12		M	UC	E4: pancolitis	0	+
UC-98	11		M	UC	E3: extensive	0	+
UC-99	10		M	UC	E1: proctitis	15	–
UC-100	3	0	F	UC	E4: pancolitis	0	–
UC-101	10		F	UC	E4: pancolitis	40	+
UC-102	9		F	UC	E3: extensive	N/A	+
UC-103	12		F	UC	E4: pancolitis	0	+
UC-104	6		M	UC	N/A	0	+
UC-105	13		F	UC	E4: pancolitis	N/A	+
UC-106	11		M	UC	E3: extensive	N/A	+
UC-107	3	0	F	UC	E4: pancolitis	N/A	+
UC-108	8		M	UC	E4: pancolitis	N/A	+
UC-109	10		F	UC	N/A	N/A	+
UC-110	13		M	UC	E1: proctitis	0	+
UC-111	9		M	UC	E4: pancolitis	0	+
UC-112	4		F	UC	E4: pancolitis	N/A	+
UC-113	10		F	UC	E4: pancolitis	10	+
UC-114	5		F	UC	E4: pancolitis	0	+

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 Anti-Integrin $\alpha v \beta 6$ Antibody in Pediatric UC 1097.e8

Supplementary Table 1. Continued

Sample	Age		Sex	Diagnosis	Extent of disease ¹	PUCAI	Anti-integrin $\alpha v \beta 6$ antibody
	y	mo					
UC-115	8		F	UC	E4: pancolitis	0	+
UC-116	13		F	UC	E4: pancolitis	35	+
UC-117	3	0	M	UC	E4: pancolitis	25	+
UC-118	9		M	UC	N/A	20	+
UC-119	9		M	UC	E4: pancolitis	0	+
UC-120	7		M	UC	E4: pancolitis	25	+
UC-121	7		M	UC	E4: pancolitis	0	+
UC-122	12		F	UC	E4: pancolitis	50	+
UC-123	14		F	UC	E4: pancolitis	20	+
UC-124	13		M	UC	E4: pancolitis	0	+
UC-125	13		M	UC	E4: pancolitis	45	+
UC-126	9		F	UC	E4: pancolitis	15	+
UC-127	4		F	UC	E4: pancolitis	60	+
UC-128	8		F	UC	E4: pancolitis	N/A	+
UC-129	1	6	M	UC	E4: pancolitis	40	+
UC-130	13		M	UC	E4: pancolitis	0	+
UC-131	9		M	UC	E2: left-side colitis	0	+

Sera of patients with UC were used to examine the subclasses and isotypes of the anti-integrin $\alpha v \beta 6$ antibody and to purify immunoglobulin (Ig) G to analyze the blocking activity against integrin $\alpha v \beta 6$ -fibronectin binding. F, female; M, male; N/A, not available; PUCAI, Pediatric Ulcerative Colitis Activity Index.

Supplementary Table 2. Characteristics of Patients With Crohn's Disease (CD)

Sample	Age, y	Age, m	Sex	Diagnosis	PCDAI	Anti-integrin $\alpha v \beta 6$ antibody
CD-1	10		M	CD	0	–
CD-2	10		M	CD	0	–
CD-3	11		M	CD	0	–
CD-4	14		F	CD	45	–
CD-5	12		M	CD	45	–
CD-6	10		M	CD	0	–
CD-7	9		F	CD	2.5	+
CD-8	11		M	CD	0	–
CD-9	10		M	CD	0	–
CD-10	13		M	CD	0	–
CD-11	8		M	CD	0	+
CD-12	15		F	CD	2.5	+
CD-13	12		M	CD	0	–
CD-14	12		M	CD	0	–
CD-15	14		M	CD	0	–
CD-16	6		M	CD	0	–
CD-17	14		M	CD	0	–
CD-18	12		M	CD	5	+
CD-19	13		F	CD	15	–
CD-20	13		F	CD	0	–
CD-21	12		M	CD	0	–
CD-22	12		F	CD	0	–
CD-23	11		M	CD	5	+
CD-24	14		M	CD	0	–
CD-25	14		F	CD	0	–
CD-26	2	11	F	CD	0	–
CD-27	14		M	CD	0	–
CD-28	12		M	CD	0	–
CD-29	13		M	CD	0	–
CD-30	4		M	CD	0	+
CD-31	14		M	CD	0	–
CD-32	9		M	CD	0	–
CD-33	9		M	CD	0	–
CD-34	12		F	CD	0	–
CD-35	13		M	CD	0	–
CD-36	12		F	CD	0	–
CD-37	7		F	CD	0	–
CD-38	3	0	M	CD	5	–
CD-39	5		M	CD	5	+

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 Anti-Integrin $\alpha v \beta 6$ Antibody in Pediatric UC 1097.e10

Supplementary Table 2. Continued

Sample	Age, y	Age, m	Sex	Diagnosis	PCDAI	Anti-integrin $\alpha v \beta 6$ antibody
CD-40	9		F	CD	0	–
CD-41	10		F	CD	0	–
CD-42	9		M	CD	0	–
CD-43	12		M	CD	0	–
CD-44	12		F	CD	0	–
CD-45	13		M	CD	47.5	–
CD-46	11		F	CD	0	+
CD-47	14		M	CD	27.5	–
CD-48	7		F	CD	0	–
CD-49	10		M	CD	45	+
CD-50	12		M	CD	17	–
CD-51	12		M	CD	15	–
CD-52	14		M	CD	82.5	–
CD-53	14		F	CD	50	+
CD-54	11		F	CD	0	+
CD-55	7		F	CD	N/A	+
CD-56	13		M	CD	5	–
CD-57	7		M	CD	5	+
CD-58	13		F	CD	0	+
CD-59	6		M	CD	0	–
CD-60	11		M	CD	N/A	+
CD-61	10		F	CD	0	+
CD-62	10		M	CD	0	+
CD-63	5		F	CD	0	+
CD-64	11		F	CD	7.5	–
CD-65	0	11	F	CD	0	+
CD-66	7		F	CD	0	+
CD-67	3	0	F	CD	22.5	+
CD-68	1	11	F	CD	20	–
CD-69	5		M	CD	20	+
CD-70	13		F	CD	30	+
CD-71	11		M	CD	5	–
CD-72	9		M	CD	N/A	–
CD-73	3	0	F	CD	5	–
CD-74	7		M	CD	25	–
CD-75	2	8	M	CD	0	–
CD-76	7		F	CD	25	+
CD-77	6		M	CD	N/A	+

Supplementary Table 2. Continued

Sample	Age, y	Age, m	Sex	Diagnosis	PCDAI	Anti-integrin $\alpha v \beta 6$ antibody
CD-78	12		F	CD	17.5	–
CD-79	9		M	CD	0	–
CD-80	10		F	CD	2.5	–
CD-81	12		M	CD	0	+
CD-82	11		M	CD	0	–
CD-83	7		F	CD	15	+
CD-84	0	5	F	CD	17.5	–
CD-85	8		M	CD	0	+
CD-86	9		M	CD	0	–
CD-87	12		F	CD	0	–
CD-88	11		M	CD	10	+
CD-89	14		F	CD	N/A	+
CD-90	6		F	CD	5	–
CD-91	8		F	CD	5	+
CD-92	2	0	F	CD	0	–
CD-93	10		M	CD	5	–
CD-94	4		F	CD	10	–
CD-95	14		F	CD	55	–

Sera of patients with CD were used to examine the subclasses and isotypes of the anti-integrin $\alpha v \beta 6$ antibody and to purify immunoglobulin (Ig) G to analyze the blocking activity against integrin $\alpha v \beta 6$ –fibronectin binding. F, female; M, male; N/A, not available; PCDAI, Pediatric Crohn's Disease Activity Index.

Supplementary Table 3. Detailed Clinical Characteristics of Patients With Crohn's Disease (CD)

	Endoscopic and pathologic findings ^a												Location	Behavior	Perianal disease	Change of diagnosis from UC	Anti-integrin $\alpha v \beta 6$ antibody			
	Typical UC findings						Typical CD findings											Paris classification ¹		
	1	2	3	4	5	Total	1	2	3	4	5	Total								
CD-1	-	-	-	-	-	0	+	+	+	-	+	4	L3	B1	+	-	-			
CD-2	-	-	-	-	-	0	+	+	+	-	+	4	L3	B1	+	-	-			
CD-3	-	-	-	-	-	0	+	+	+	-	+	4	L3	B1	+	-	-			
CD-4	-	-	-	-	-	0	+	+	+	-	+	4	L3+L4a+L4b	B1	N/A	-	-			
CD-5	-	-	-	-	-	0	+	+	+	-	+	4	L3	B1	+	-	-			
CD-6	-	-	-	-	-	0	-	-	+	-	+	2	L3	B1	+	-	-			
CD-7	-	-	-	-	-	0	+	-	+	-	-	2	L3+L4a+L4b	B1	-	-	+			
CD-8	-	-	-	-	-	0	+	+	+	-	-	3	L3	B1	-	-	-			
CD-9	-	-	-	-	-	0	+	+	+	+	-	4	L3	B1	+	-	-			
CD-10	-	-	-	-	-	0	-	-	+	-	+	2	L3+L4b	B1	+	-	-			
CD-11	-	-	-	-	-	0	+	+	+	-	-	3	L3+L4a	B1	+	-	+			
CD-12	+	+	-	+	+	4	+	+	-	-	-	2	L2+L4b	B2	-	+	+			
CD-13	-	-	-	-	-	0	-	+	+	-	+	3	L3+L4a+L4b	B1	+	-	-			
CD-14	-	-	-	-	-	0	+	-	+	-	-	2	L3+L4b	B1	+	-	-			
CD-15	-	-	-	+	-	1	-	-	+	-	-	1	L3+L4a	B1	+	-	-			
CD-16	-	-	-	-	-	0	-	-	+	-	-	1	L4a+L4b	B2	-	-	-			
CD-17	-	-	-	+	-	1	+	+	+	-	+	4	L3+L4a	B1	+	-	-			
CD-18	-	-	-	-	-	0	+	+	+	-	-	3	L3+L4a+L4b	B1	+	-	+			
CD-19	-	-	-	-	+	1	+	+	+	-	+	4	L3+L4a	B2	+	-	-			
CD-20	-	-	-	-	+	1	+	+	+	-	+	4	L3+L4a	B1	+	-	-			
CD-21	-	-	-	-	+	1	+	-	-	-	+	2	L1	B1	+	+	-			
CD-22	-	-	-	-	+	1	+	+	+	-	+	4	L3+L4a+L4b	B1	+	-	-			
CD-23	-	-	-	-	+	1	+	-	+	-	+	3	L3+L4a	B1	-	-	+			
CD-24	-	-	-	-	+	1	-	-	+	-	-	1	L1+L4a	B1	+	-	-			
CD-25	-	-	-	+	+	2	-	-	+	-	-	1	L3	B3	+	-	-			
CD-26	-	-	-	-	+	1	+	+	+	-	-	3	L1+L4a+L4b	B1	+	+	-			
CD-27	-	-	-	-	+	1	+	+	+	-	+	4	L1+L4a+L4b	B1	-	-	-			
CD-28	-	-	-	-	+	1	+	+	+	-	+	4	L3+L4a+L4b	B2	+	-	-			
CD-29	-	-	-	-	+	1	-	-	+	-	-	1	L3+L4a+L4b	B1	+	-	-			
CD-30	-	-	-	-	+	1	-	-	+	-	+	2	L3+L4a+L4b	B1	+	-	+			
CD-31	-	-	-	-	+	1	+	+	+	-	+	4	L3+L4a+L4b	B1	-	-	-			
CD-32	-	-	-	-	+	1	+	+	+	-	+	4	L3+L4a	B1	+	-	-			
CD-33	-	-	-	-	+	1	-	-	+	-	+	2	L3+L4a+L4b	B1	+	-	-			
CD-34	-	-	-	-	-	0	+	+	+	-	-	3	L2	B1	-	-	-			
CD-35	-	-	-	-	+	1	+	+	+	-	+	4	L3+L4a+L4b	B1	-	-	-			
CD-36	-	-	-	-	+	1	+	+	+	-	-	3	L3+L4a	B1	-	-	-			

Supplementary Table 3. Continued

	Endoscopic and pathologic findings ^a													Change of diagnosis from UC	Anti-integrin $\alpha v \beta 6$ antibody		
	Typical UC findings						Typical CD findings						Paris classification ¹				
	1	2	3	4	5	Total	1	2	3	4	5	Total	Location			Behavior	Perianal disease
CD-37	-	-	-	-	+	1	+	+	+	-	+	4	L3	B1	+	-	-
CD-38	-	-	-	-	-	0	-	-	+	-	+	2	L2	B1	+	-	-
CD-39	-	-	-	-	-	0	+	+	+	-	-	3	L4b	B1	-	-	+
CD-40	-	-	-	-	-	0	-	-	+	-	+	2	L1	B1	+	-	-
CD-41	-	-	-	-	-	0	+	+	+	+	+	5	L1	B1	+	-	-
CD-42	-	-	-	-	-	0	+	+	+	-	-	3	L1	B1	-	-	-
CD-43	-	-	-	-	-	0	-	-	+	+	+	3	L3+L4a	B1	+	-	-
CD-44	-	-	-	-	-	0	+	+	+	+	+	5	L3	B1	+	-	-
CD-45	-	-	-	-	-	0	+	+	+	-	+	4	L3	B2	-	-	-
CD-46	-	-	-	-	-	0	+	+	+	-	+	4	L3	B1	-	-	+
CD-47	-	-	-	-	-	0	+	+	+	-	-	3	L4a+L4b	N/A	N/A	-	-
CD-48	-	-	-	-	-	0	+	-	-	-	-	1	L1	B2	-	-	-
CD-49	+	+	+	-	+	4	-	-	-	-	-	0	L2+L4a	B1	-	+	+
CD-50	-	-	-	-	-	0	+	+	+	-	-	3	L3+L4b	B1	-	-	-
CD-51	-	-	-	-	-	0	+	+	+	+	+	5	L3	B1	-	-	-
CD-52	-	-	-	-	+	1	+	+	+	-	+	4	L3	B1	-	-	-
CD-53	+	+	+	+	+	5	-	-	-	-	+	1	L3+L4a	B1	-	-	+
CD-54	+	+	+	+	+	5	-	-	-	+	-	1	L3	B1	-	+	+
CD-55	+	+	+	+	+	5	-	-	-	-	-	0	L3+L4a+4b	B2	+	+	+
CD-56	-	-	-	-	+	1	-	-	+	+	-	2	L3+ L4b	B1	+	-	-
CD-57	+	+	+	+	+	5	-	-	-	-	-	0	L3+L4a+L4b	B1	-	+	+
CD-58	+	+	+	+	+	5	-	+	-	-	+	2	L3+L4a+L4b	B1	-	+	+
CD-59	-	-	+	-	+	2	-	-	+	+	+	3	L3+L4a+L4b	B1	+	-	-
CD-60	+	-	+	+	+	4	+	+	+	-	+	4	L2	B1	-	-	+
CD-61	-	-	+	+	+	3	-	-	+	-	-	1	L3+L4a+L4b	B1	-	-	+
CD-62	-	-	+	+	+	3	-	-	-	-	-	0	L2+L4a	B1	-	+	+
CD-63	+	+	+	+	+	5	-	+	-	-	+	2	L2+L4a	B1	-	-	+
CD-64	-	-	-	+	+	2	-	-	+	+	+	3	L3+L4a+L4b	B1	+	-	-
CD-65	+	+	+	+	+	5	-	-	-	-	+	1	L3+L4a+L4b	B1	-	+	+
CD-66	+	-	+	+	+	4	+	+	+	-	+	4	L2+L4a+L4b	B1	-	-	+
CD-67	+	+	+	+	+	5	-	-	-	-	+	1	L3+L4a	B1	-	-	+
CD-68	-	-	-	-	+	1	+	-	+	+	+	4	L3	B1	+	-	-
CD-69	+	+	+	+	+	5	-	-	+	-	-	1	L3+L4a+L4b	B1	-	+	+
CD-70	+	+	-	+	+	4	+	-	+	-	-	2	L3	B1	-	+	+
CD-71	-	-	-	-	-	0	-	-	+	-	-	1	L3+L4a+L4b	B1	-	-	-
CD-72	-	-	+	+	+	3	-	+	+	-	-	2	L3+L4a+L4b	B1	-	-	-

Supplementary Table 3. Continued

	Endoscopic and pathologic findings ^a													Change of diagnosis from UC	Anti-integrin $\alpha v\beta 6$ antibody		
	Typical UC findings						Typical CD findings						Paris classification ¹				
	1	2	3	4	5	Total	1	2	3	4	5	Total	Location			Behavior	Perianal disease
CD-73	-	-	-	+	+	2	+	-	+	+	+	4	L3	B1	+	-	-
CD-74	-	-	-	-	-	0	-	-	+	-	-	1	L1+L4a+L4b	B1	-	-	-
CD-75	-	-	-	-	+	1	-	-	+	-	-	1	L2+L4a	B1	-	-	-
CD-76	+	+	+	+	+	5	-	-	+	-	-	1	L3+L4a+L4b	B1	-	+	+
CD-77	+	+	+	+	+	5	-	+	-	-	-	1	L3+L4a	B1	-	+	+
CD-78	+	-	+	+	+	4	+	+	+	+	-	4	L3+L4a+L4b	B1	+	-	-
CD-79	-	-	-	-	+	1	-	-	+	+	+	3	L1+L4a+L4b	B1	+	-	-
CD-80	-	-	-	-	-	0	-	-	+	+	+	3	L1	B1	+	-	-
CD-81	-	+	-	-	+	2	+	-	+	-	-	2	L3+L4a+L4b	B1	-	-	+
CD-82	-	-	-	-	-	0	-	-	+	+	+	3	L3+L4a+L4b	B1	+	-	-
CD-83	+	+	+	+	+	5	+	-	+	+	+	4	L3+L4a+L4b	B1	+	-	+
CD-84	-	-	-	-	+	1	+	-	+	-	+	3	L3+L4a+L4b	B1	-	-	-
CD-85	+	+	+	+	+	5	-	-	+	-	-	1	L3+L4a+L4b	B1	-	+	+
CD-86	-	-	-	-	+	1	+	+	+	+	-	4	L3+L4a+L4b	B1	+	-	-
CD-87	-	-	-	-	-	0	+	+	+	+	+	5	L3+L4a+L4b	B1	+	-	-
CD-88	+	+	+	+	+	5	+	+	+	-	-	3	L3+L4a+L4b	B1	-	-	+
CD-89	-	-	-	-	-	0	+	+	+	-	+	4	L3	B1	-	-	+
CD-90	-	-	-	-	-	0	-	-	+	-	+	2	L3+L4a+L4b	B1	-	-	-
CD-91	-	-	-	-	-	0	+	+	+	-	+	4	L3+L4a	B2	+	-	+
CD-92	-	-	-	-	-	0	+	-	+	-	-	2	L1	B1	-	-	-
CD-93	-	-	-	-	-	0	+	+	+	-	+	4	L3	B1	+	-	-
CD-94	-	-	-	-	-	0	+	-	+	-	-	2	L3+L4a+L4b	B1	-	-	-
CD-95	-	-	-	-	-	0	+	+	+	-	-	3	L3	B1	-	-	-

Endoscopic findings were evaluated by pediatricians or gastroenterologists blinded for the anti-integrin $\alpha v\beta 6$ antibody titer. We classified various endoscopic and pathologic findings of the patients with CD into typical UC and typical CD findings as previously reported (Supplementary Figure 1D).^{2,3} The total number of these findings was calculated for each patient. B1, nonstricturing, non-penetrating; B2, stricturing; B3, penetrating; B2B3, both penetrating and stricturing disease; either at the same time or at different times; L1, distal 1/3 ileum \pm limited cecal disease; L2, colonic; L3, ileocolonic; L4, isolated upper disease (L4a, upper disease proximal to ligament of Treitz; L4b, upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum); UC, ulcerative colitis.

^aFindings in 5 samples.

Supplementary References

1. Levine A, et al. *Inflamm Bowel Dis* 2011;17:1314–1321.
2. Levine A, et al. *J Pediatr Gastroenterol Nutr* 2014;58:795–806.
3. Oliveira SB, et al. *BMJ* 2017;357:1–15.